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(54) Title: AMPLIFICATION OF THE VITAMIN B_{12} UPTAKE SYSTEM USING POLYMERS

(57) Abstract

The invention relates to the oral delivery of peptide and protein pharmaceuticals using the vitamin B_{12} (VB_{12}) uptake system, with the delivery being amplified using polymers. More particularly, the invention concerns a complex having the general formula: $(V - Q)_n$-P - $(Q' - A)_m$, where V is a carrier which will bind to natural intrinsic factor (IF) selected from vitamin B_{12} or an analogue thereof, n is the molar substitution ratio of V in the complex, and is a number from 1.0 to about 10, P is a pharmaceutically acceptable polymer, A is a pharmaceutically active substance, m is the molar substitution ratio of A in the complex, and is a number greater than 1.0 to about 1000, Q and Q' are independently a covalent bond, or a spacer compound linking V, P and A by covalent bonds.

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AMPLIFICATION OF THE VITAMIN B_{12} UPTAKE SYSTEM USING POLYMERS

BACKGROUND OF THE INVENTION

The invention relates to the oral delivery of peptide and protein pharmaceuticals using the vitamin B_{12} (VB_{12}) uptake system. More particularly the invention relates to the amplification of the uptake system using polymers.

The oral route of administration of peptides such as LHRH and its analogues, or proteins such as Granulocyte Colony Stimulating Factor (GCSF), Erythropoietin (EPO) and insulin, as pharmaceuticals in the treatment of systemic conditions has in the past met with little success. In general, the amount of peptide required for successful oral administration has been 100 to 1000 times the dose required for parenteral delivery, thus making the administration of these agents via this route prohibitively expensive. There are two fundamental reasons for the lack of success. Firstly, the intestinal milieu has a high degree of proteolytic activity, which rapidly degrades most peptides. Secondly, while there are well defined uptake mechanisms for individual amino acids and di-peptides, there is no general mechanism for polypeptides to be transported across the membrane of the mucosal epithelium into the circulation. Rather, this membrane is designed as a general barrier prohibiting the uptake of the numerous foreign proteins encountered in this environment. Thus, although a peptide may be modified to withstand the enzymatic barrage encountered in the intestine, such modification is of little value if the peptide cannot subsequently cross the mucosal barrier and enter the systemic circulation.

Recent work by the current inventor, which is described in PCT Patent Application PCT/AU86/00299 (WO87/02251), has however provided a method to overcome the mucosal barrier. This method takes advantage of the natural intrinsic factor (IF) mediated uptake mechanism for vitamin B_{12} (VB_{12}). VB_{12} is a naturally occurring

dietary molecule which is actively taken up from the intestine. During this process it first binds to intrinsic factor (IF) in the upper small intestine. The [VB_{12}-IF] complex passes down the small intestine and binds to an IF receptor located on the surface of the ileal epithelium. The whole [VB_{12}-IF-Receptor] complex is then internalized by receptor-mediated endocytosis and some time later the VB_{12} appears in serum.

PCT Application PCT/AU86/00299 (WO87/02251) describes methods to modify chemically VB_{12} to provide suitable functional groups for conjugation of the VB_{12} to various drugs and peptide/protein pharmaceuticals. When the [VB_{12}-pharmaceutical] complex is administered orally it is possible to utilise the natural IF-mediated VB_{12}-uptake system to deliver the pharmaceutical to the circulation.

One major limitation to this general VB_{12} uptake mechanism is that the dose of pharmaceutical which can be delivered per feed to the recipient is low. The dose is directly proportional to the amount of VB_{12} which can be taken up per feed. Thus, in mice and rats it is only possible to deliver around 20-40 pMoles of pharmaceutical per dose, while in humans the quantity of pharmaceutical which can be delivered is approximately 1 nMole. While this level of uptake is sufficient to deliver pharmaceutically active doses of some substances, such as LHRH agonists, calcitonin and EPO, it is not sufficient to deliver proteins such as GCSF and insulin at quantities large enough to have a pharmacological effect. It would therefore be desirable to amplify the uptake capacity of the VB_{12} transport system, by at least 10 fold.

Polymers have been proposed for the administration of active agents. Polymers with pendant groups linked to the backbone via spacers containing aromatic diazo bonds are known. Many of these polymers have been designed specifically to release the pendant side groups following cleavage of the diazo bond by azo-reductases released by bacteria in the colon. These polymers however have proven to be unsuitable for delivering drugs systemically following oral administration, because these polymer-drug complexes are

not absorbed intact from the intestine, but rather deliver their drug to the colon, following cleavage of the diazo bond by colonic enzymes. Small amounts of the released drug may eventually reach the circulation, but the amount which reaches the circulation has been found to be of little practical value.

Polymers to which are conjugated various cytotoxic drugs have also been proposed. These polymers have been targeted to cancer cells using specific antibodies or sugar moieties. Once the drug-polymer has reached its target tissue the complex is endocytosed by the target cell and the pendant drug is released by the action of lysosomal enzymes, or by cleavage of a disulfide linked drug by intracellular glutathione. Oral delivery of such complexes has not, however, resulted in significant uptake of the drug-polymer complex from the intestinal lumen into the circulation.

SUMMARY OF THE INVENTION

It is the object of this invention to describe a new set of drug/pharmaceutical-polymer conjugates, to which a VB_{12} molecule, or analogue or derivative thereof, has been conjugated. These VB_{12}-polymer-drug conjugates are suitable for oral delivery, as they can utilize the aforementioned VB_{12}-transport system for uptake and have the added advantage of increasing the amount of pharmaceutical agent which can be delivered via the VB_{12} uptake mechanism.

A complex having the general formula

$$(V - Q)_n - P - (Q' - A)_m$$

wherein, V is a carrier which will bind to natural intrinsic factor (IF) selected from vitamin B_{12} or an analogue thereof;

n is the molar substitution ratio of V in the complex, and is a number from 1.0 to about 10;

P is a pharmaceutically acceptable polymer;

A is a pharmaceutically active substance;

m is the molar substitution ratio of A in the complex, and is a number greater than 1.0

to about 1000;

Q and Q' are independently a covalent bond, or a spacer compound linking V, P and A by covalent bonds.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The polymer, P, of the present invention can be any pharmaceutically acceptable polymer. The polymer is able to attach to at least one carrier molecule and to at least one, but preferably a multiplicity of active substance molecules.

The polymer P may be a biodegradable polymer, such as a biodegradable carbohydrate polymer, or a polymer of amino acids. Otherwise, the polymer may be a non-biodegradable polymer, in which case it preferably has attached biodegradable side chains allowing for covalent linkage to an active substance.

Suitable polymers for substitution with VB_{12} and modification according to the invention, include poly[N-(2-hydroxypropyl)-methacrylamide], dextran, chondroitan sulfate, water soluble polyurethanes formed by covalent linkage of PEG with lysine, poly(glutamic acid), poly(hydroxypropyl-glutamine) and branched chaim polypeptides formed by the dual modification of the α- and ϵ-amino groups of lysine during the peptide synthesis. Such polymers may have multiple amino-termini, to which can be conjugated a plurality of the pharmaceutical or drug to be delivered. The polymers can also be formed with multiple cysteines, to provide free thiols, or multiple glutamates or aspartates, to provide free carboxyls for conjugation using suitable carbodiimides. Similarly the polymer can contain multiple histidines or tyrosines for conjugation. When the polymer is a branched chain polypeptide, it may also be further modified to provide multiple functional groups for coupling to the active substance.

Some examples of suitable polymers are polysaccharides, including dextran, inulin, cellulose, starch and derivatives thereof; chondroitan sulfate, poly[N-(2-

hydroxypropyl)-methacrylamide] and derivatives thereof, styrene-maleic anhydride copolymer, divinylether-maleic anhydride copolymer, polylysine, poly(glutamic acid), poly(hydroxypropyl glutamine), poly(lactic acid), water-soluble polyurethanes formed by covalent linkage of PEG with lysine or other amino acids and branched chain polypeptides.

If the polymer is a branched chain polypeptide, the polymer may in one preferred form have the general sequence:
$(R^5_{16}\text{-}Lys_8\text{-}R^4_8\text{-}Lys_4\text{-}R^3_4\text{-}Lys_2\text{-}R^2_2\text{-}Lys)_n\text{-}R^1\text{-}COOH$ where n is from 1 to 17, R^1 is any sequence of from 1 to 10 amino acids, R^2, R^3, R^4, and R^5 are each independently any sequence of from 0 to 6 amino acids, providing that not all of R^2, R^3, R^4, and R^5 are a sequence of 0 amino acids, and where the polymer may terminate at any position within the brackets. Some examples of polymers having this general formula include the polypeptides with the sequence $(Gly_4\text{-}Lys_2\text{-}Ser_2\text{-}Lys)_5\text{-}Ala\text{-}COOH$ and $(Gly_{16}\text{-}Lys_8\text{-}Lys_4\text{-}His_4\text{-}Glu_4\text{-}Lys_2\text{-}Lys)\text{-}Gly_5\text{-}Cys\text{-}COOH$, for example. The terminal amino groups on the end amino acids may be further chemically modified to bond with the active substances, if desired.

The carrier, V, is derived from vitamin B_{12} (VB_{12}) or an analogue of vitamin B_{12}, which will bind to natural intrinsic factor (IF). The carrier may also be chemically modified in order to bond with the polymer.

Suitable analogues of VB_{12} for derivatization prior to conjugation to the polymer include any variant or derivative of VB_{12} (cyanocobalamin) which possesses binding activity to intrinsic factor. Preferred analogues of VB_{12} also include aquocobalamin, adenosylcobalamin, methylcobalamin, hydroxycobalamin, cyanocobalamin, carbanalide, and 5-methoxybenzylcyanocobalamin [(5-MeO)CN-Cbl] as well as the desdimethyl, monoethylamide and the methylamide analogues of all of the above. Other analogues include all alkyl cobalamins in which the alkyl chain is linked to the corrin nucleus by

a direct CoC covalent bond. Other analogues include chlorocobalamin, sulfitocobalamin, nitrocobalamin, thiocyanatocobalamin, benzimidazolecyanocobalamin derivatives such as the: 5,6-dichlorobenzimidazole, 5-hydroxybenzimidazole, trimethylbenzimidazole, as well as adenosylcyanocobalamin [(Ade)CN-Cbl], cobalamin lactone, cobalamin lactam and the anilide, ethylamide, monocarboxylic and dicarboxylic acid derivatives of VB_{12} or its analogues.

Preferred derivatives of VB_{12} also include the mono-, di- and tricarboxylic acid derivatives or the propionamide derivatives of VB_{12}. Carriers may also include analogues of VB_{12} in which the cobalt is replaced by zinc or nickel. The corrin ring of VB_{12} or its analogues may also be substituted with any substituent which does not effect its binding to IF, and such derivatives of VB_{12} or its analogues are part of this invention. Other derivatives of VB_{12} or its analogues which have a functional group which is able to react with the spacer compound are also part of the invention. Other derivatives and analogues of vitamin B_{12} are discussed in Schneider, Z. and Stroinski, A.: *Comprehensive B_{12}*; (Walter De Gruyter; Berlin, NY; 1987), the disclosure of which is incorporated herein by reference.

The pharmaceutically active substance, A, is any suitable pharmaceutical substance especially a biologically active polypeptide, or a part of this peptide. For example it may be a hormone, growth factor, interleukin, cytokines, lymphokines, or similar substances. Some preferred active substances include GCSF, EPO, LHRH, interferon, or biologically active analogues, parts or derivatives of these substances, calcitonin, TRH, vasopressin, oxytocin, insulin, Growth Hormone, somatostatin, GM-CSF, SCGF, (stem cell growth factor), CGRP or biologically active analogues, parts of derivatives of the above.

Examples of typical substances for delivery according to the invention include active substances such as hormones and bioactive peptides (and analogues and derivatives

thereof) such as LHRH, Vasopressin, oxytocin, Insulin, testosterone, interferon, somatotrophin, somatostatin, Erythropoietin, Colony Stimulating factors (GCSF, GM-CSF, CSF), PMSG, HCG, Inhibin, PAI-2: therapeutic agents such as neomycin, salbutamol, pyrimethamine, penicillin G, methicillin, carbenicillin, pethidine, xylazine, ketamine HC1, mephenesin, GABA, iron dextran, nucleotide analogues or ribozyme.

Further examples of active substances include polypeptides such as insulin, somatostatin, somatostatin derivatives (U.S. Pat. Nos. 4,087,390, 4,093,575, 4,100,117 and 4,253,398), growth hormones, prolactin, adrenocorticotropic hormone (ACTH), melanocyte stimulating hormone (MSH), thyroid hormone releasing hormone (TRH), its salts, and derivatives thereof (U.S. Pat. Nos. 3,957, 247 and 4,100,152), thyroid stimulating hormone (TSH), luteinizing hormone (LH), follicle stimulating hormone (FSH), vasopressin, vasopressin derivatives [desmopressin [Folia Endocrinologica Japonica 54, No. 5, p. 676-691 (1978)]], oxytocin, calcitonin, parathyroid hormone, glucagon, gastrin, secretin, pancreozymin, cholecystokinin angiotensin, human placental lactogen, human chorionic gonadotropin (HCG), enkephalin, enkephalin derivatives [U.S. Pat. No. 4,277, 394, European patent application Publication No. 31567], endorphin, kyotorphin, interferons (α, β, γ), interleukins (I, II, and III), tuftsin, thymopoietin, thymosin, thymostimulin, thymic humoral factor (TFH), serum thymic factor (FTS), and its derivatives (U.S. Pat. No. 4,229,438) and other thymic factors [Medicine in Progress 125, No. 10, p.835-843 (1983)], tumor necrosis factor (TNF), colony stimulating factor (CSF), motilin, dinorphin, bombesin, neurotensin, cerulein, bradykinin, urokinase, asparaginase, kallikrein, substance P analogue and antagonist, nerve growth factor, blood coagulation factors VIII and IX, lysozyme chloride, polymixin B, colistin, gramicidin, bacitracin, protein synthesis stimulating peptides (British patent No. 8232082), gastric inhibitory polypeptide (GIP), vasoactive intestinal polypeptide (VIP), platelet-derived growth factor (PDGF), growth hormone factor (GRF, somatocrinin), bone morphogenetic protein (BMP), epidermal growth factor (EGF), etc.

Examples of antitumor agents include bleomycin hydrochloride, methotrexate, actinomycin D, mitomycin C, vinblastine sulfate, vincristine sulfate, daunorubicin hydrochloride, adriamycin, neocarzinostatin, cytosine arabinoside, fluorouracil, tetrahydrofuryl-5-fluorouracil, krestin, picibanil, lentinan, levamisole, bestatin, azimexon, glycyrrhizin, poly I:C, poly A:U and poly ICLC.

Examples of antibiotics, include gentamicin, dibekacin, kanendomycin, lividomycin, tobramycin, amikacin, fradiomycin, sisomicin, tetracycline hydrochloride, oxytetracycline hydrochloride, rolitetracycline, doxycycline hydrochloride, ampicillin, piperacillin, ticarcillin, cephalothin, cephaloridine, cefotiam, cefsulodin, cefmenoxime, cefmetazole, cefazolin, cefotaxime, cefoperazone, ceftizoxime, moxolactam, latamoxef, thienamycin, sulfazecin, and azthreonam.

The aforementioned antipyretic, analgesic and antiinflammatory drugs include, for instance, sodium salicylate, sulpyrine, sodium flufenamate, sodium diclofenac, sodium indomethacin, morphine hydrochloride, pethidine hydrochloride, levorphanol tartrate and oxymorphone. Examples of the antitussives and expectorants may be mentioned ephedrine hydrochloride, methylephedrine hydrochloride, noscapine hydrochloride, codeine phosphate, dihydrocodeine, phosphate, alloclamide hydrochloride, chlophedianol hydrochloride, picoperidamine hydrochloride, cloperastine, protokylol hydrochloride, isoproterenol hydrochloride, salbutamol sulfate and terbutaline sulfate, Examples of sedatives include chlorpromazine hydrochloride, prochlorperazine, trifluoperazine, atropine sulfate and scopolamine methylbromide. The muscle relaxants include, among others, pridinol methanesulfonate, tubocurarine chloride and pancuronium bromide. The antiepileptics include, for instance, sodium phenytoin, ethosuximide, sodium acetazolamide and chlordiazepoxide hydrochloride. Examples of antiulcer drugs include metoclopramide and L-histidine monohydrochloride. Examples of antidepressants include imipramine, clomipramine, noxiptiline and phenelzine sulfate. The antiallergic drugs include, among others, diphenhydramine

hydrochloride, chlorpheniramine maleate, tripelenamine hydr chloride, methdilazine hydrochloride, clemizole hydrochloride, diphenylpyraline hydrochloride and methoxyphenamine hydrochloride. The cardiotonics include among others, trans-*p*-oxocamphor, theophyllol, aminophylline and etilefrine hydrochloride. The antiarrythmic agents include, for instance propranolol hydrochloride, alprenolol hydrochloride, bufetolol hydrochloride and oxyprenolol hydrochloride. The vasodilators include, among others, oxyfedrine hydrochloride, diltiazem hydrochloride, tolazoline hydrochloride, hexobendine and bamethan sulfate. The antihypertensive diuretics include, among others, hexamethonium bromide, pentolinium, mecamlamine hydrochloride, ecarazine hydrochloride and clonidine hydrochloride. Examples of antidiabetics include sodium glymidine, glypizide, phenformin hydrochloride, buformin hydrochloride and metformin. The anticoagulants include, among others, sodium heparin and sodium citrate.

The haemostatic agents include, among others, thromboplastin, thrombin, menadione sodium bisulfite, acetomenaphthone, ϵ-amino-caproic acid, tranexamic acid, carbazochrome sodium sulfonate and adrenochrome monoaminoguanidine methanesulfonate. Among antituberculotics are isoniazid, ethambutol and sodium *p*-aminosalicylate. The hormone drugs are exemplified by prednisolone succinate, prednisolone sodium phosphate, dexamethasone sodium sulfate, betamethasone sodium phosphate, hexestrol phosphate, hexestrol acetate and methimazole. The antinarcotic agents include, among others, levallorphan tartrate, nalorphine hydrochloride and naloxone hydrochloride.

If the active substance is GCSF, for example, it can be linked to the polymer with a spacer compound containing a disulfide bond. In this case, the disulfide bond may be formed with the buried thiol group of the cysteine at position 17 in the polypeptide chain of GCSF.

When the active substance is a LHRH analogue, it may be ANTIDE or an analogue of ANTIDE. ANTIDE is a linear decapeptide containing a number of unnatural and/or D-amino acids in addition to three L-amino acid residues. Its N-terminus is acetylated and the C-terminus amidated. ANTIDE has the following sequence:

N-Ac-D-Nal(2), D-Phe(*p*Cl), D-Pal(3), Ser, Lys(Nic), D-Lys(Nic), Leu, Lys(iPr), Pro, D-A1a-NH_2

[Nal(2) represents 3-(2-napthyl) alanine; Phe(*p*-Cl) represents 3-(4-chlorophenyl)alanine: Pal(3) represents 3-(3-pyridyl)alanine; Lys(Nic) represents *N*-nicotinoyllysine; Lys-(iPr) represents *N*-isopropyllysine].

For ANTIDE-1 the residue 6 is D-Lys, not D-Lys(Nic); for ANTIDE-2 the residue 5 is Lys, not Lys(Nic); and for ANTIDE-3 the residue 8 is Lys, not Lys(iPr).

When the active substance is a LHRH analogue, it may also be histrellin, D-Lys_6-LHRH, D-Lys_6-LHRH-ethylamide, or an analogue of these substances.

The spacer compounds Q and Q' are optional. When they are absent the carrier V and/or the active substance A are linked to polymer P by a direct covalent bond. They are introduced either to improve the intrinsic factor affinity of the VB_{12} complex or to overcome problems in the coupling of the carrier, V and/or the active substance A arising from unfavourable steric interactions between the V and/or A with the polymer P or to increase the bioactivity of A in the complex. The spacer compounds may also act as linking agents, being bi-functional compounds with selected functional groups on each end to react with suitable functional groups located on the polymer, and also the VB_{12} carrier molecule and/or on the pharmaceutically active substances.

The spacer compound Q and/or Q' preferably comprises optionally substituted saturated or unsaturated, branched or linear, C_{1-50} alkylene, cycloalkylene or aromatic group, optionally with one or more carbons within the chain being replaced with N, O

or S, and wherein the optional substituents are selected from, for example, carbonyl, carboxy, hydroxy, amino and other groups.

Suitable extended spacers for the conjugation of the pharmaceutical (A) or the carrier (V) to the polymer matrix (P) include: disuccinimidyl suberate (DSS), *bis*(sulfosuccinimidyl) suberate (BSS), ethylene glycol*bis*(succinimidylsuccinate) (EGS), ethylene glycol*bis*(sulfosuccinimidylsuccinate) (Sulfo-EGS), *p*-aminophenylacetic acid, dithio*bis*(succinimidylpropionate) (DSP), 3,3'-dithio*bis*-(sulfosuccinimidylpropionate) (DTSSP), disuccinimidyl tartarate (DST), disulfosuccinimidyl tartarate (Sulfo-DST), *bis*[2-(succinimidooxycarbonyloxy)-ethylene]sulfone (BSOCOES), *bis*[2-(sulfosuccinimidooxycarbonyloxy)-ethylene]sulfone (Sulfo-BSOCOES), dimethyl adipimidate.2HCl (DMA), dimethyl pimelimidate.2HCl (DMP), dimethyl suberimidate.2HCl (DMS).

Suitable cross-linking agents for use in the preparation of thiol-cleavable biodegradable spacers or linkers include *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), iminothiolane,sulfosuccinimidyl-6-[3-(2-pyridyldithio)propionamido]-hexanoate(Sulfo-LC-SPDP), succinimidyl-6-[3-(2-pyridyldithio)propionamido]-hexanoate (LC-SPDP), sulfosuccinimidyl 6-[α-methyl-α-(2-pyridyldithio)toluamido]-hexanoate (Sulfo-LC-SMPT), 1,4-di[3'-(2'-pyridyldithio)propionamido]-butane (DPDPB), 4-succinimidyloxycarbonyl-α-methyl-α-(2-pyridyldithio)-toluene (SMPT), dimethyl 3,3'dithiobispropionimidate.2HCl (DTBP).

In the formula, n, the average molar substitution ratio of V in the complex, is a number from 1.0 to about 10. Preferably n is between 1.0 and 1.2. The polymer is ideally linked to one V carrier molecule, but as the polymer is of uncertain size and/or structure, the number n represents a statistical average. Also, in the formula, m, the average molar substitution ratio of A in the complex, represents a number from at least 1.0 to about 1000. In order to amplify the uptake of the active ingredient m should be

as large as possible, ideally between about 10 to 100. m is also a statistical average, and different numbers of active molecules (A) will be bound to the polymer, because of the variation in the polymeric structure of P.

The invention concerns a complex which comprises more than one active substance linked to a polymer, which is linked to at least one carrier molecule which is a VB_{12} molecule, or analogue thereof, wherein the ability of the carrier to undergo the binding reactions necessary for uptake and transport of the VB_{12} in a vertebrate host and the activity of the active substance are substantially maintained, following conjugation or following biological release of the active substance from the polymer. The complex, having the general formula
$(V - Q)_n - P - (Q' - A)_m$ where V, Q, P, Q', A, n and m are as defined previously, is prepared by one of the following methods:

a) reacting A with P to form an intermediate complex, and thereafter reacting the intermediate complex with A;

b) reacting V with P to form an intermediate complex and thereafter reacting the intermediate complex with A;

c) the process of step a) or b) wherein one or more of V, P or A are modified to provide at least one functional group capable of forming a chemical linkage prior to coupling with the other reactants; or

d) reacting one or two V, P or A with Q and/or Q' prior to coupling with the other reactants.

In general terms, the process may comprise one or more of the following steps:

a) reacting the active substance with the polymer to form said complex;

b) chemically modifying the active substance to provide at least one functional group capable of forming a chemical linkage, and reacting the active substance

and polymer to form said complex;

c) chemically modifying the carrier to provide at least one functional group capable of forming a chemical linkage and reacting the carrier and polymer to form said complex;

d) chemically modifying the active substance and the polymer to provide functional groups capable of forming a chemical linkage, and reacting the active substance and polymer to form said complex;

e) reacting the active substance with at least one cross-linking agent and reacting the active substance with the polymer to form said complex;

f) reacting the carrier with at least one cross-linking agent and reacting the polymer and carrier to form said complex;

g) reacting the active substance and polymer with at least one cross-linking agent and reacting the active substance and polymer to form said complex;

h) reacting the active substance directly with a polymeric support to form an intermediate containing a plurality of molecules of the active substance linked to the polymer, and subsequently coupling to the polymer-active substance intermediate one or more carrier molecules;

i) coupling at least one carrier molecules directly to a polymeric support to form an intermediate containing at least one molecule of the carrier linked to the polymer, and subsequently reacting the active substance to the polymer-carrier intermedia to a plurality of active substance molecules.

The invention also provides a method for the modification of a polymeric support to introduce functional groups capable of reacting either directly with the active substance or with a chemically-modified form of the active substance. The resulting polymer-active substance intermediate contains one or more molecules of the active substance, said intermediate being suitable for coupling to the carrier to give a complex capable of amplified delivery of the active substances.

In one embodiment of the invention the linkage joining the pharmaceutical, or the carrier to the polymer is a disulfide bond. In a further embodiment of the invention the linkage joining the pharmaceutical, or the carrier to the polymer is an ester linkage. In yet another embodiment of the invention the linkage joining the pharmaceutical or the carrier to the polymer is a γ-glutamyl-ϵ-lysine bond. In yet another embodiment of the invention the linkage joining the pharmaceutical or the carrier to the polymer is a diazo-linkage. In a preferred embodiment of the invention there is a complex comprising multiple molecules of GCSF linked through a disulfide bond by reaction with a (dithiopyridyl-propionamido)dodecylamine- derivative of the polymer. In another preferred embodiment of the invention there is a complex comprising multiple molecules of GCSF linked through a disulfide bond to a (dithiopyridyl-propionamido)-dodecylsuberylhexyl-derivative of the polymer.

It has been found that it is possible to synthesize three analogues of ANTIDE (LHRH antagonist) which are suitable for conjugation to a polymer matrix, namely:
N-Ac-D-Nal(2), D-Phe(*p*Cl), D-Pal(3), Ser, Lys(Nic), D-Lys, Leu, Lys(iPr), Pro, D-Ala-NH_2 (D-Lys_6ANTIDE or ANTIDE-1);
N-Ac-D-Nal(2), D-Phe(*p*Cl), D-Pal(3), Ser, Lys, D-Lys(Nic), Leu, Lys(iPr), Pro, D-Ala-NH_2 (Lys5-ANTIDE or ANTIDE-2); and
N-Ac-D-Nal(2), D-Phe(*p*Cl), D-Pal(3), Ser, Lys(Nic), D-Lys(Nic), Leu, Lys, Pro, D-Ala-NH_2 (Lys_8ANTIDE or ANTIDE-3).

The invention also concerns a pharmaceutical composition which comprises a complex as described previously together with a pharmaceutically acceptable carrier or excipient as are well known in the art and described, for example, in Remington's Pharmaceutical Sciences (Mack Publishing Company, 10th Edition, which is incorporated herein by reference). The composition may be in the form of a capsule, tablet, slow release dosage form, elixir, gel, paste, or enterically coated dosage form, for example, or any other suitable dosage form as is well known in the art.

Complexes and compositions according to this invention may be administered to a human or animal subject, optionally in association with one or more carriers and/or excipients. Modes of administration are not critical to this invention and include parenteral (intraveneous, intramuscular, or intraorgan injection), oral, transdermal, vaginal, anal, or other administration routes as are well known in the art. In the context of the treatment of diseases, a therapeutically effective amount of a complex or compound according to this invention is that which provides treatment of a particular disease state. What constitutes an effective amount will depend upon the nature of the disease being treated, the consulting physician or veterinary surgeon judgement, and other factors such as the age, weight and/or sex of the subject. By way of example only, an effective amount of a complex composition according to the invention may comprise from one nanogram to 10 grams of a complex in accordance with the invention. The present invention is applicable to the treatment of any disease state which is responsive to the administration of peptide pharmaceuticals.

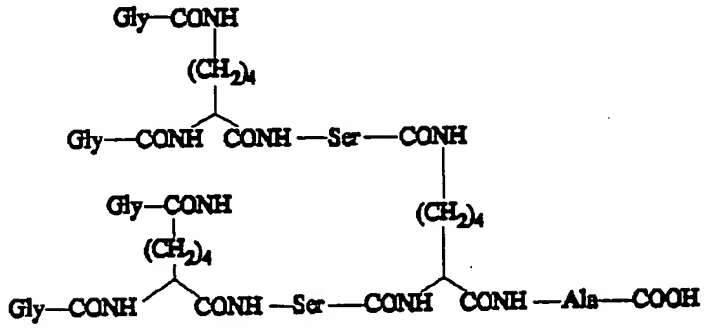
A method for the treatment of disease is also part of the invention, which comprises administering to a subject a therapeutically effective amount of a complex as described above, preferably in form of a pharmaceutical composition.

This invention in a further aspect relates to the use of complexes described herein for the manufacture of medicaments, and for the administration thereof to humans and animals.

EXAMPLE 1: Synthesis of Multi-Lysine polymer 1 (MLP1)

A multi-Lysine polymer (MLP1) of the general formula
$(Gly_4\text{-}Lys_2\text{-}Ser_2\text{-}Lys]_5\text{-}Ala\text{-}COOH$,
was synthesized on an Applied Biosystems peptide synthesiser. More precisely this can be represented as $(Gly_4\text{-}Lys_2\text{-}Ser_2\text{-}Lys)_4\,(Gly_4\text{-}Lys_2\text{-}Ser_2\text{-}Lys)\text{-}Ala\text{-}COOH$.

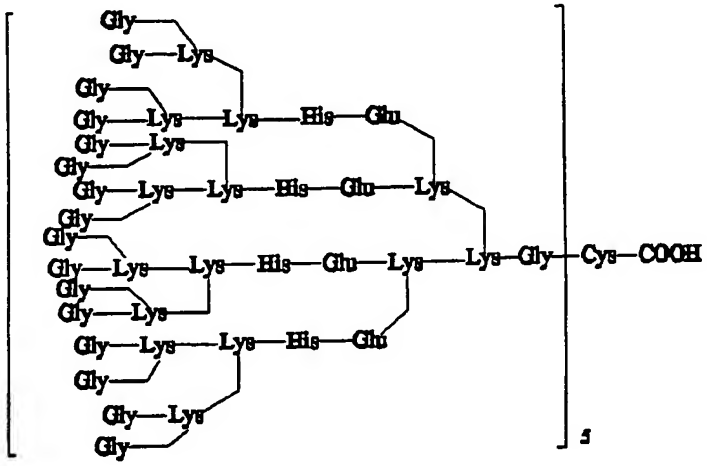
The formula of $(Gly_4\text{-}Lys_2\text{-}Ser_2\text{-}Lys)_5\text{-}Ala\text{-}COOH$ can be represented as follows:



which shows the structure more precisely. If necessary the terminal amino-groups on the glycine can be further chemically modified.

EXAMPLE 2: Synthesis of Multi-Lysine polymer 2 (MLP2)

A multi-Lysine polymer (MLP2) of the general formula $(Gly_{16}\text{-}Lys_8\text{-}Lys_4\text{-}His_4\text{-}Glu_4\text{-}Lys_2\text{-}Lys)\text{-}Gly_5\text{-}Cys\text{-}COOH$ was synthesized on an Applied Biosystems peptide synthesiser. More precisely the structure can be represented as follows:



EXAMPLE 3: Production of adipyl-hydrazide derivative of eVB_{12} carboxylate

The adipyl-hydrazide derivative of eVB_{12} carboxylate was prepared for conjugation to the terminal carboxyl groups of the polymer by reaction with EDAC. The adipyl-hydrazide derivatives used can be represented by the following (shorthand) chemical structure:

adipyl-hydrazido-eVB_{12} (= eVB_{12}-$CONHNHCO(CH_2)_4CONHNH_2$)

This reagent was readily prepared in one step from eVB_{12} carboxylate by the addition of EDAC to a mixture of the acid and a 20-fold excess of adipylhydrazide; ie:

$$eVB_{12}CO_2H \xrightarrow[\text{EDAC}]{\text{20eq. adipyl hydrazide}} eVB_{12}CONHNHCO(CH_2)_4CONHNH_2$$

EXAMPLE 4: Formation of Polymer-ANTIDE conjugates using non-cleavable homobifunctional cross-linkers.

Previous experiments have shown that the direct conjugation between ANTIDE-1 and ANTIDE-3 and VB_{12} produced conjugates with greatly reduced bioactivity when compared to ANTIDE. The close proximity of the VB_{12} to ANTIDE using this conjugation strategy presumably sterically interferes with the binding of ANTIDE to the LHRH receptor. In order to reduce the steric effect possibly seen with direct conjugation, bifunctional, non-biodegradable linkers, must be used to produce covalent complexes between the two polymers and ANTIDE-1 and 3. As an example, ANTIDE-1 or ANTIDE-3 were reacted with a 1.5 molar excess of Disuccinimidyl suberate (DSS) for 10 minutes at Room Temperate (RT). MLP1 or MLP2 was then added and the reaction allowed to proceed overnight. Conjugated material was purified by chromatography on Sephadex G-25 in 10% acetic acid, followed by Reversed Phase HPLC (RP-HPLC). The Polymer-Anilido-ANTIDE-1 and ANTIDE-3 conjugates were formed by reaction of MLP 1 or MLP 2 with *p*-aminophenylacetic acid using

EDAC/NHS. The Polymer-anilide, was in turn conjugated to ANTIDE-1 and ANTIDE-3 using DSS. The conjugated material was purified by G-25 chromatography in 10% acetic acid followed by RP-HPLC.

eVB_{12} was linked to the polymer ANTIDE complexes by reacting adipyl-hydrazidyl-"e"VB_{12} with the complex using EDAC. The reacted product was purified by RP-HPLC.

EXAMPLE 5: Formation of MLP-ANTIDE conjugates using thiol-cleavable cross-linkers.

Conjugates were also prepared in which the covalent linker contained a biodegradable disulfide bond, which would be reduced *in vivo*, presumably by glutathione in serum. Briefly, MLP1 or MLP2 was reacted with N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP). The dithiopyridyl-MLP (DTP-MLP) product was purified by RP-HPLC. A free thiol was introduced onto ANTIDE-1 by reaction with SPDP. The dithiopyridyl group was then reduced with mercapto-ethanol and the product purified by RP-HPLC. Similarly, a free thiol was introduced into ANTIDE-3 by reaction with iminothiolane. The thiolated product ($SH\text{-}HN^{+}$ANTIDE-3) was purified by RP-HPLC. Formation of the disulfide linked MLP-ANTIDE-1 and MLP-ANTIDE-2 conjugates was achieved by reaction of the thiolated ANTIDE derivative with DTP-MLP in 2.5% acetic acid for 24 hours. The conjugated material was purified by Sephadex G-25 chromatography, followed by RP-HPLC.

VB_{12} was linked to the polymer-ANTIDE complexes by reacting a[illegible] "e"VB_{12} with the complex using EDAC. The reacted prod[illegible]gates using HPLC.

EXAMPLE 6: Formation of VB_{12}-AN[illegible]

thiol-cleavable cross-linkers.

It is desirable to increase the amount of drug, or its analogues, which can be taken up by the VB_{12}-transport system, by the linkage of multiple copies of the drug to a polymeric backbone, to which is conjugated one or more VB_{12} molecules. One process is described for the formation of such a VB_{12}-drug-polymer complex using iminothiolated ANTIDE-1 or DLEA (SH-HN*ANTIDE-1/DLEA), a thiolated VB_{12}-derivative and DTP-Lysyl-polyglutamate. A second process is described for the formation of a VB_{12}-drug-polymer complex in three steps by (i) converting the polysaccharide dextran to a poly(aminohexyl) dextran (ii) reacting this material with a small amount of VB_{12} succinimidyl ester and a large excess of SPDP to form a [DTP-hexyl]$_x$-dextran-[hexyl-VB_{12}]$_y$ derivative (where $x >> y$) and (iii) reacting this material with a thiolated peptide, in these examples iminothiolated ANTIDE-1 or DLEA, to form a [Peptide-dithiohexyl]$_z$-dextran-[hexyl-VB_{12}]$_y$ derivative (where $z > y$).

a) Formation of DTP-Lysyl-polyglutamate

Poly-glutamate (100 mg) (MW 64,600-70,000; Sigma), was reacted with EDAC (100 mg) and NHS (50 mg in acetone) for 10 minutes at RT. Lysine (400 mg in 4 ml 1% $NaHCO_3$) was added and allowed to react overnight (O/N). The product, Lysyl-Polyglutamate (LPG), was purified by extensive dialysis against DW, and then lyophilised.

The dithiopyridyl derivative of LPG, was obtained by reacting Dithiopyridyl-propionic acid (50 mg) with O-(N-Succinimidyl)-N,N,N'N'-tetramethyluronium tetrafluoroborate (TSTU) (100 mg) and N-Ethyldiisopropyl amine (100 mg; DIEA) in DMF for 1 hour. The succinimidyl ester so formed was added directly to 100 mg LPG dissolved in 4 ml 2% $NaHCO_3$. The reaction was allowed to proceed O/N after which the product (DTP-LPG) was purified by exhaustive dialysis against DW, and then lyophilised.

b) Preparation of Iminothiolated ANTIDE-1

ANTIDE-1 (20 mg) was dissolved in 300 ul 5% DIEA in DMF containing 5 mg EDTA plus 5 mg DTT, which had been degassed under argon. Iminothiolane (20 mg in 50 ul DIEA/DMF plus 50 ul Borate buffer, 100 mM pH 8.2) was added and reacted for 60 minutes before purification by RP-HPLC and lyophilisation.

c) Preparation of Iminothiolated aminoethyl-VB_{12}

Aminoethyl"e"VB_{12} (20 mg) was dissolved in 300 ul 5% DIEA in DMF containing 5 mg EDTA plus 5 mg DTT, which had been degassed under argon. Iminothiolane (20 mg in 50 ul DIEA/DMF plus 50 ul Borate buffer, 100 mM pH 8.2) was added and reacted for 60 minutes before purification by RP-HPLC and lyophilisation.

d) Preparation of Iminothiolated DLEA

DLEA (10 mg) was dissolved in 300 ul 5% DIEA in DMF containing 5 mg EDTA plus 5 mg DTT, which had been degassed under argon. Iminothiolane (20 mg in 50 ul DIEA/DMF plus 50 ul Borate buffer, 100 mM pH 8.2) was added and reacted for 60 minutes before purification by RP-HPLC and lyophilisation.

e) Formation of VB_{12}-ANTIDE-Lysyl-Polyglutamate.

DTP-LPG was dissolved at 20 mg/ml in DW. Iminothiolated-aminoethyl-VB_{12} was dissolved at 5 mg/ml in DW and added to the DTP-LPG (1:20 w/w) and allowed to react for 20 min at RT before the addition of iminothiolated-ANTIDE-1 (50 mg/ml in DW) dropwise with stirring. The reaction mixture was kept at pH 6.5-7.0 by the addition of Tris.HCL pH 7.0 and NaAcetate, pH 5.5. The reaction proceeded overnight

after which the product was purified by dialysis and then lyophilised. The composition of the product was determined by amino acid analysis and found to contain 1 : 5 : 21, ANTIDE-1 : Lysine : Glutamate, or roughly 25 % by weight ANTIDE.

f) Formation of VB_{12}-DLEA-Lysyl-Polyglutamate.

DTP-LPG was dissolved at 20 mg/ml in DW. Iminothiolated-aminoethyl-VB_{12} was dissolved at 5 mg/ml in DW and added to the DTP-LPG (1:20 w/w) and allowed to react for 20 min at RT before the addition of iminothiolated-DLEA-1 (50 mg/ml in DW) dropwise with stirring. The reaction mixture was kept at pH 6.5-7.0 by the addition of Tris.HCL pH 7.0 and NaAcetate, pH 5.5. The reaction proceeded overnight after which the product was purified by dialysis and then lyophilised. The composition of the product was determined by amino acid analysis and found to contain 1 : 3: 16, DLEA-1 : Lysine : Glutamate, or roughly 28 % by weight DLEA.

g) Formation of poly(aminohexyl)-dextran

A solution of 2 g of dextran (Dextran T70, MW 70,000, Pharmacia) in 20 ml of distilled water was stirred at room temperature and a solution of sodium periodate (2.4 g) in water (25 ml) was added. The reaction mixture was stirred for 4 h and excess periodate was removed by addition of 500 μl of glycerol. The reaction mixture was dialyzed for 24 h at 4° C against 2 x 5 l of MilliQ-filtered water. The retentate was lyophilised to give 1.8 g of oxidised dextran as a white powder. A sample of this material (250 mg) was taken up with gentle heating in 10 ml of acetate buffer (200 mM, pH 5). 1,6-diaminohexane (5 ml of 100 mg/ml, pH 7 solution) was added and the solution was stirred for 3h at room temperature. Sodium cyanoborohydride (2 x 100 mg) was added the reaction mixture was left for 1 h. Finally sodium borohydride (100 mg) was added. After a total reaction time of 5 h the solution was transferred to dialysis tubing and dialysed at 4° C against 2 x 2 l of MilliQ-filtered water the retentate

was lyophilised to give 180 mg of poly(aminohexyl)-dextran.

Analysis of the amine content of the product by TNBS assay gave a 14% amine content (= % moles of amine per mole of glucose), which corresponds to approximately fifty free amine groups per polymer chain.

If the procedure described above was repeated using 1 g of dextran and 2 g of sodium periodate in the initial oxidation step the amine content of the final product was 32%.

If the procedure described above was repeated using 125 mg of oxidised dextran and 4 ml of 100 mg/ml 1,6-diaminohexane solution in the second step the amine content of the final product was 24%.

If the procedure described above was repeated only using sodium borohydride in the second step the amine content of the final product was 11%.

h) Formation of [DTP-hexyl]$_x$-dextran-[hexyl-NHCO-VB$_{12}$]$_y$

Prep 1: A sample of poly(aminohexyl)-dextran (50 mg, 14% amine content) was taken up in 3 ml of borate buffer (100 mmol, pH 8) and dioxane (1 ml). The solution was stirred at room temperature and a sample of the N-hydroxysuccinimidyl ester of eVB$_{12}$carboxylate (0.5 mg) in water (100 μl) was added. After fifteen minutes a solution of SPDP (9 mg) in 100 μl of dioxane was added. The reaction mixture was stirred for 60 minutes at room temperature and unreacted SPDP was destroyed by the addition of 200 μl of 1M ethylenediamine solution. The modified polymer was separated from other reagents by size-exclusion chromatography on G-25 Sephadex, eluting with 5% acetic acid. The product fractions were combined and lyophilised to give 42 mg of

VB_{12}/DTP-modified dextran as a pale pink powder.

Prep 2: A polymer more heavily substituted with VB_{12} was prepared using poly(aminohexyl)-dextran (20 mg; 12% amine content), which was taken up in 1 ml of bicarbonate buffer (100 mmol, pH 9.5) and dioxane (100 μl). The solution was stirred at room temperature and SPDP (2 x 3.5 mg) in 100 μl of dioxane was added. After 60 minutes the N-hydroxysuccinimidyl ester of eVB_{12}carboxylate (1 x 0.8 mg, 1 x 1.6 mg) in water (100 μl) was added in two aliquots. The reaction mixture was stirred for 60 minutes at room temperature and unreacted NHS-ester was destroyed by the addition of a few drops of ethylenediamine solution. The modified polymer was separated from other reagents by size-exclusion chromatography on G-25 Sephadex, eluting with 5% acetic acid. The product fractions were combined and lyophilised to give 13 mg of VB_{12}/DTP-modified dextran as a dark red powder.

i) Formation of [Antide1-dithiohexyl]$_x$ dextran-[hexyl-VB_{12}]$_y$

A solution of the [DTP-hexyl]$_x$-dextran-[hexyl-NHCO-VB_{12}]$_y$ polymer (Prep 1, 15 mg) was taken up in 200 mM/pH 4 acetate buffer (3 ml). A crystal of Na_2EDTA was added and the solution was deoxygenated with argon. A solution of 5 mg of iminothiolated ANTIDE-1 in water (100 μl) was added and the reaction mixture was stirred for 30 minutes. A further 2 x 10 mg of thiolated peptide in water (100 μl) were added and the solution was stirred for 16 h at room temperature. The peptide-conjugated polymer was separated from other reagents by size-exclusion chromatography on G-25 Sephadex, eluting with 5% acetic acid. The product fractions were combined and lyophilised to give 19.5 mg of VB_{12}/DTP-modified dextran as a faint pink powder. The peptide content of the polymer was found by amino acid analysis to be 20% by weight, and the VB_{12} content was found by U.V. analysis and by intrinsic factor binding assay to be 1% by weight.

(j) Formation of [DLEA-dithiohexyl]$_x$ dextran-[hexyl-VB$_{12}$]$_y$

A solution of the [DTP-hexyl]$_x$-dextran-[hexylNHCO-VB$_{12}$]$_y$ polymer (Prep 2, 10 mg) was taken up in 200 mM/pH 4 acetate buffer (2.5 ml), a crystal of Na_2EDTA was added and the solution was deoxygenated with argon. A solution of 5 mg of iminothiolated DLEA in water (100 μl) was added and the reaction mixture was stirred for 30 minutes. A further 5 mg of thiolated peptide in water (100 μl) was added and the solution was stirred for 16 h at room temperature. The peptide-conjugated polymer was separated from other reagents by size-exclusion chromatography on G-25 Sephadex, eluting with 5% acetic acid. The product fractions were combined and lyophilised to give 5 mg of VB$_{12}$/DTP-modified dextran as pink powder. The peptide content was found to be 14% by weight by amino acid analysis, and the VB$_{12}$ content was found to be 2% by U.V. analysis and by intrinsic factor binding assay.

THE CLAIMS:

1. A complex having the general formula

$$(V - Q)_n - P - (Q' - A)_m$$

wherein, V is a carrier which will bind to natural intrinsic factor (IF) selected from vitamin B_{12} or an analogue thereof;

n is the molar substitution ratio of V in the complex, and is a number from 1.0 to about 10;

P is a pharmaceutically acceptable polymer;

A is a pharmaceutically active substance;

m is the molar substitution ratio of A in the complex, and is a number greater than 1.0 to about 1000;

Q and Q' are independently a covalent bond, or a spacer compound linking V, P and A by covalent bonds.

2. The complex according to claim 1, wherein at least one of Q and Q' is a spacer compound which contains a biodegradable portion.

3. The complex according to claim 2 wherein said biodegradable portion is selected from a disulfide bond, ester linkage, γ-glutamyl-ϵ-lysine linkage, or a diazo bond.

4. The complex according to claim 1, wherein n is from 1.0 to about 1.2 and m is from 2 to about 200.

5. The complex according to claim 1, wherein n is from 1.0 to about 1.2, and m is from 10 to 100.

6. A complex according to claim 1 wherein said P is a biodegradable polymer.

7. A complex according to claim 6 wherein said biodegradable polymer is selected from a biodegradable carbohydrate polymer or a polymer of amino acids.

8. A complex according to claim 1 wherein said polymer is non-biodegradable.

9. A complex according to claim 8 wherein said non-biodegradable polymer has attached biodegradable side chains for covalent linkage to an active substance.

10. A complex according to claim 1 wherein said polymer is selected from: the polysaccharides comprising dextran, inulin, cellulose, starch and derivatives thereof; chondroitan sulfate, poly[N-(2-hydroxypropyl)-methacrylamide] and derivatives thereof; styrene-maleic anhydride copolymer; divinylether-maleic anhydride copolymer; polylysine, poly(glutamic acid), poly(hydroxypropyl glutamine); poly(lactic acid); water-soluble polyurethanes formed by covalent linkage of PEG with lysine or other amino acids; and branched chain polypeptides.

11. A polymer according to claim 10 wherein said polymer is a branched chain polypeptide optionally modified to provide multiple functional groups for coupling of an active substance.

12. A complex according to claim 11 wherein said polymer has the sequence:
$(R^5_{16}\text{-Lys}_8\text{-}R^4_8\text{-Lys}_4\text{-}R^3_4\text{-Lys}_2\text{-}R^2_2\text{-Lys})_n\text{-}R^1\text{-COOH}$
where n is from 1 to 17, R^1 is any sequence of from 1 to 10 amino acids, R^2, R^3, R^4, and R^5 are each independently any sequence of from 0 to 6 amino acids, providing that not all of R^2, R^3, R^4, and R^5 are a sequence of 0 amino acids, and where the polymer may terminate at any position within the brackets.

13. A complex according to claim 12 wherein said polymer has the sequence of

$(Gly_4\text{-}Lys_2\text{-}Ser_2\text{-}Lys)_5$-Ala-COOH.

14. A complex according to claim 12 wherein said polymer has the sequence $(Gly_{16}\text{-}Lys_8\text{-}Lys_4\text{-}His_4\text{-}Glu_4\text{-}Lys_2\text{-}Lys)\text{-}Gly_5$-Cys-COOH.

15. A complex according to claim 10 wherein said polymer is poly[N-2(2-hydroxypropyl)-methacrylamide].

16. A complex according to claim 1 wherein said spacer compound Q or Q' comprises optionally substituted saturated or unsaturated, branched or linear, C_{1-50} alkylene, cycloalkylene or aromatic group, optionally with one or more carbons within the chain being replaced with N, O or S, and wherein the optional substituents are selected from carbonyl, carboxy, hydroxy, amino and other groups.

17. A complex according to claim 16 wherein said spacer compound is derived from disuccinimidyl suberate (DSS), *bis*(sulfosuccinimidyl) suberate (BSS), ethylene glycol*bis*(succinimidylsuccinate) (EGS), ethylene glycol*bis*(sulfosuccinimidylsuccinate) (sulfo-EGS), *p*-aminophenylacetic acid, dithio*bis*(succinimidylpropionate) (DSP), 3,3-'diothi*bis*(sulfosuccinimidyl tartarate (sulfo-DST), *bis*[(2-succinimidooxycarbonyloxy)-ethylene]sulfone (BSOCOES), *bis*[2-(sulfosuccinimidooxycarbonyloxy)-ethylene]sulfone (sulfo-BSOCOES), *bis*-(sulfosuccinimidooxycarbonyloxy)-ethylene]sulfone (sulfo-BSOCOES), dimethyl adipimidate.2HC1 (DMAA), dimethyl pimelimidate.HC1 (DMP and dimethyl suberimidate.2HC1 (DMS).

18. A complex according to claim 16 wherein said spacer compound is thio-cleavable.

19. A complex according to claim 18 wherein said thiol-cleavable spacer is derived from *N*-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), iminothiolane, sulfosuccinimidyl-6-[3-(2-pyridyldithio)-propionamido]-hexanoate (sulfo-LC-SPDP), succinimidyl-6-[3-(2-pyridyldithio)-propionamido] hexanoate (LC-SPDP), sulfosuccinimidyl-6-[α-methyl-α-(2-pyridyldithio)-toluamido]-hexanoate (sulfo-LC-SMPT), 1,4-di[3'-(2'-pyridyldithio)priopionamido]-butane (DPDPB), 4-succinimidyl-oxycarbonyl-α-methyl-α-(2-pyridyldithio)toluene (SMPT) and dimethyl-3,3'dithio*bis*propionimidate.2HC1 (DTBP).

20. A complex according to claim 1 wherein said active substance A is a biologically active polypeptide or a part thereof.

21. A complex according to claim 20 wherein said polypeptide is a hormone, growth factor, interleukin.

22. A complex according to claim 20 wherein said polypeptide is selected from LHRH or interferon or a part thereof or an analogue thereof.

23. A complex according to claim 22 wherein said LHRH analogue is histrellin, or an analogue of histrellin.

24. A complex according to claim 22 wherein said LHRH analogue is D-Lys$_6$-LHRH.

25. A complex according to claim 22 wherein said LHRH analogue is D-Lys$_6$-LHRH-ethylamide.

26. A complex according to claim 22 wherein said LHRH analogue is ANTIDE or an analogue of ANTIDE.

27. A complex according to claim 26 wherein said ANTIDE analogue is D-Lys_6-ANTIDE.

28. A complex according to claim 26 wherein said ANTIDE analogue is Lys_8-ANTIDE.

29. A complex according to claim 1 wherein said VB_{12} carrier is selected from cyanocobalamin, aquocobalamin, adenosylcobalamin, methylcobalamin, hydroxycobalamin, cyanocobalamin carbanalide, 5-methoxylbenzylcobalamin, and the desdimethyl, monoethylamide and methylamide analogues of all of the preceding analogues, as well as coenzyme B12, 5'-deoxyadenosylcobalamin, chlorocobalamin, sulfitocobalamin, nitrocobalamin, thiocyanatocobalamin, 5,6-dichlorobenzimadazole, 5-hydroxybenzimidazole, trimethylbenzimidazole, adenosylcyanocobalamin, cobalaminlactone, cobalamin lactam and the analide; ethylamide, propionamide; monocarboxylica and dicarboxylic acid derivatives of VB_{12} or its analogues; or alkyl cobalamins in which the alkyl chain is linked to the carrier nucleus by a direct CoC covalent bond.

30. A complex according to claim 1 wherein the carrier is a vitamin B_{12} analogue in which the Co is replaced by Ni or Zn.

31. A complex according to claim 29 wherein the carrier is a vitamin B_{12} analogue in which the corrin ring is substituted with a substituent which does not effect binding to intrinsic factor.

32. A process for the production of a complex having the general formula

$$(V - Q)_n - P - (Q' - A)_m$$

wherein V, Q, P, Q', A, n and m are as defined in claim 1, said process selected from:

a) reacting A with P to form an intermediate complex, and thereafter reacting the intermediate complex with V;

b) reacting V with P to form an intermediate complex and thereafter reacting the intermediate complex with A;

c) the process of step a) or b) wherein one or more of V, P or A are modified to provide at least one functional group capable of forming a chemical linkage prior to coupling with the other reactants; or

d) reacting one or two V, P or A with Q and/or Q' prior to coupling with the other reactants.

33. A process according to claim 32 wherein Q and/or Q' comprises an optionally substituted saturated or unsaturated, branched or linear, C_{1-50} alkylene, cycloalkylene or aromatic group, optionally with one or more carbons within the chain being replaced with N, O or S, and wherein the optional substituents are selected from carbonyl, carboxy, hydroxy, amino and other groups.

34. A process according to claim 32 wherein Q' is a cleavable cross-linking agent containing a disulfide bond.

35. A process according to claim 32 wherein the cross-linking agents are selected from disuccinimidyl suberate (DSS), *bis*(sulfosuccinimidyl) suberate (BSS), ethylene glycol*bis*(succinimidylsuccinate) (EGS), ethylene glycol*bis*(sulfosuccinimidylsuccinate) (Sulfo-EGS), *p*-amino-phenylacetic acid, dithio*bis*(succinimidylpropionate) (DSP), 3,3'-dithio*bis*(sulfosuccinimidylpropionate) (DTSSP), disuccinimidyl tartarate (DST), disulfosuccinimidyl tartarate (Sulfo-DST), *bis*[2-(succinimidyloxycarbonyloxy)-

ethylene]sulfone (BSOCOES), *bis*[2-(sulfosuccinimidooxycarbonyloxy)-ethylene]sulfone (Sulfo-BSOCOES), dimethyl adipimidate.2 HCl (DMA), dimethyl pimelimidate.2HCl (DMP), dimethyl suberimidate.2HCl (DMS).

36. A process according to claim 32 wherein said spacer is selected from disuccinimidyl suberate (DSS), *bis*(sulfosuccinimidyl) suberate (BSS), ethylene glycol*bis*(succinimidylsuccinate) (EGS), ethylene glycol*bis*(sulfosuccinimidylsuccinate) (Sulfo-EGS), p-amino-phenylacetic acid, dithio*bis*(succinimidylpropionate) (DSP), 3,3'-dithio*bis*(sulfosuccinimidylpropionate) (DTSSP), disuccinimidyl tartarate (DST), disulfosuccinimidyl-tartarate (Sulfo-DST), *bis*[2-(succinimidyloxycarbonyloxy)-ethylene]sulfone (BSOCOES), *bis*[2-(sulfosuccinimidooxycarbonyloxy)-ethylene]-sulfone (Sulfo-BSOCOES), dimethyl adipimidate.2HCl (DMA), dimethyl pimelimidate.2HCl (DMP), dimethyl suberimidate.2HCl (DMS).

37. A process according to claim 32 wherein said spacer is selected from N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), iminothiolane, sulfosuccinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (Sulfo-LC-SPDP), succinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (LC-SPDP), sulfosuccinimidyl 6-[-methyl--(2-pyridyldithio) toluamido]hexanoate (Sulfo-LC-SMPT), 1,4-di[3'-(2'-pyridyldithio)propionamido]butane (DPDPB), 4-succinimidyloxycarbonyl-α-methyl-α-(2-pyridyldithio)-toluene (SMPT), dimethyl 3,3'dithiobispropionimidate.2HCl (DTBP).

38. A process according to claim 32 wherein the cross-linking agents are selected from *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), iminothiolane, sulfosuccinimidyl 6-[3-(2-pyridyldithio) propionamido]-hexanoate (Sulfo-LC-SPDP), succinimidyl 6-[3-(2-pyridyldithio) propionamido]-hexanoate (LC-

SPDP), sulfosuccinimidyl 6-[α-methyl-α-(2-pyridyldithio)-toluamido]hexanoate (Sulfo-LC-SMPT), 1,4-di[3'-(2'-pyridyldithio)propionamido]butane (DPDPB), 4-succinimidyloxycarbonyl-α-methyl-α-(2-pyridyldithio)-toluene (SMPT), dimethyl 3,3'dithio*bis*propionimidate.2HCl (DTBP).

39. A composition which comprises a complex according to claim 1 together with a pharmaceutically or agriculturally acceptable carrier or excipient.

40. A composition according to claim 39 in the form of a capsule, tablet, slow release dosage form, elixir, gel, paste, or enterically coated dosage form.

41. A method for the treatment of disease which comprises administering to a subject a therapeutically effective amount of a complex according to claim 1, or a pharmaceutical composition according to claim 39.

42. Use of a complex as defined in any one of claims 1 to 10 or 12 to 31 for the manufacture of a medicament.

43. Use of a complex as defined in any one of claims 1 to 10 or 12 to 31 for the administration to a human or animal subject.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 94/00273

A. CLASSIFICATION OF SUBJECT MATTER
Int. Cl.[5] A61K 47/48, 31/68, 37/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K 47/48 : VIT B12, INTRINSIC FACTOR, PEPTIDE UPTAKE POLYMER

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)
WPAT, CASM

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	WO 92/17167 (BIOTECH AUSTRALIA PTY LTD) 15 October 1992 (15.10.92) whole specification	1-5, 8-10, 20-29, 32, 39, 40
Y	WO 90/04606 (ROYAL FREE HOSPITAL OF MEDICINE) 3 May 1990 (03.05.90) whole specification	1-10
Y	WO 93/06767 (LA JOLLA CANCER RESEARCH FOUNDATION 28 June 1990 (28.06.90) whole specification	1-10

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search
10 August 1994 (10.08.94)

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
Y	WO 93/25221 (ALKERMES CONTROLLED THERAPEUTICS, INC.) 23 December 1993 (23.12.93) whole specification	1-10
Y	US 5206219 (APPLIED ANALYTICAL INDUSTRIES, INC.) 27 April 1993 (27.04.93)	1-10
Y	WO 87/02251 (BIOTECHNOLOGY AUSTRALIA PTY LTD)	1-15
A	DE,A, 2546474 (ISRAEL, MURRAY) 21 April 1977 (21.04.77)	1-43

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	9217167	AU	15580/92	CA	20841945	EP	531497
WO	9004606	EP	439508	GB	8824591		
WO	9306767	US	5188094				
WO	9325221	AU	46308/93				
US	5206219	US	5206219				
WO	8702251	AU	65289/86	CA	1330791	EP	220030
DE	2546474	DE	2546474				

END OF ANNEX